ab102890 – HRP
Conjugation Kit Protocol

Antibody and protein modification

View kit datasheet: www.abcam.com/ab102890
(use www.abcam.cn/ab102890 for China, or www.abcam.co.jp/ab1092890 for Japan)

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

Abcam’s HRP Conjugation Kit provides a simple and quick process to conjugate your primary antibodies with HRP.

The conjugated antibody can be used straight away in WB, ELISA, IHC etc

The antibody to be labelled should be purified, in an appropriate buffer for conjugation and at a suitable concentration, as described in the protocol booklet. If not, consider using our antibody purification and concentration kits. [http://www.abcam.com/kits/antibody-purification-and-concentration-kits](http://www.abcam.com/kits/antibody-purification-and-concentration-kits)

The Kit is available in 5 sizes

- 30µg (3 x 10 µg) sufficient for labeling up to 30µg of antibody
- 100µg (1 x 100 µg) sufficient for labeling up to 100µg of antibody
- 300µg (3 x 100µg) sufficient for labeling up to 300µg of antibody
- 1mg (1 x 1mg) sufficient for labeling up to 1mg of antibody
- 5mg (5 x 1mg) sufficient for labeling up to 5mg of antibody
Kit Contents

<table>
<thead>
<tr>
<th></th>
<th>3 x 10µg Kit</th>
<th>1 x 100µg Kit</th>
<th>3 x 100µg Kit</th>
<th>1 x 1 mg Kit</th>
<th>5 x 1 mg Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quencher</td>
<td>1 vial</td>
<td>1 vial</td>
<td>1 vial</td>
<td>1 vial</td>
<td>1 vial</td>
</tr>
<tr>
<td>Modifier</td>
<td>1 vial</td>
<td>1 vial</td>
<td>1 vial</td>
<td>1 vial</td>
<td>1 vial</td>
</tr>
<tr>
<td>HRP Mix</td>
<td>3 x 10µg</td>
<td>1 x 100 µg</td>
<td>3 x 100 µg</td>
<td>1 x 1 mg</td>
<td>5 x 1 mg</td>
</tr>
</tbody>
</table>

2. Reagents Required, Not supplied

- Unconjugated antibody or protein.

3. Storage and Handling

Kits are shipped at ambient temperature in a tamper-evident polypropylene container. Store at -20°C upon receipt.
4. Recommended antibody quantities

The amount of antibody used for labeling ideally should correspond to molar ratios between 1:1 and 1:4 Ab to HRP.

The amount of antibody used for labeling should ideally correspond to mass ratios between 1:4 and 1:1 Antibody to HRP. This means for that for 1 mg of antibody you need to add between 1-4 mg of HRP. Antibody concentrations in the range 0.5-5 mg/ml generally give optimal results, but concentrations and volumes outside these suggested ranges have also yielded active conjugates. The maximum antibody volume for each differing amounts of antibody is shown in the table below.

<table>
<thead>
<tr>
<th>Amount of antibody for 1:4 Ab:HRP (µg)</th>
<th>Amount of antibody for 1:1 Ab:HRP (µg)</th>
<th>Maximum antibody conjugation volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µg</td>
<td>40 µg</td>
<td>10 µL</td>
</tr>
<tr>
<td>100 µg</td>
<td>400 µg</td>
<td>100 µL</td>
</tr>
<tr>
<td>1 mg</td>
<td>4 mg</td>
<td>1 mL</td>
</tr>
<tr>
<td>5 mg</td>
<td>20 mg</td>
<td>5 mL</td>
</tr>
</tbody>
</table>
5. Pre-conjugation considerations

The purified antibody to be labeled should ideally be in 10-50mM amine-free buffer (e.g. MES, MOPS, HEPES, PBS), pH range 6.5 to 8.5. If the buffer is more concentrated or outside this pH range contact the technical support team.

Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA), and sugars have no effect on conjugation efficiency. We do not recommend performing a HRP conjugation in presence of sodium azide or adding sodium azide with your HRP conjugated antibody. Glycerol up to 50% has no effect.

Avoid buffer components that are nucleophilic, as these may react with chemicals. Primary amines (e.g. amino acids or ethanolamine) and thiols (e.g. mercaptoethanol or DTT) fall within this class (Note: Tris-based buffers should be avoided). If your buffer contains primary amines and/or thiols, you should consider using our Concentration and Purification Kits.
Antibody concentration and purification kit selection guide:

Has your antibody been purified from serum / ascites fluid / cell culture supernatant?

- **Yes**
  - Does the buffer contain BSA or gelatin?
    - **Yes**
      - Antibody concentration is below 1mg/ml
        - Antibody purification kits:
          - ab102764 (Rabbit)
          - ab128745 (Mouse)
          - ab128747 (Rat, Sheep, Human and Goat)
    - **No**
      - The buffer contains Tris, azide or glycine
      - Antibody concentration kits:
        - ab102778
  - **No**
    - What material is your antibody in?
      - Tissue culture supernatant
        - Antibody TCS purification kits:
          - ab128749 (Mouse)
          - ab109207 (Rabbit and Human)
      - Serum or ascites fluid
        - Antibody serum purification kits:
          - ab109209 (Rabbit)
          - ab128751 (Rat, Sheep, Human, Goat and Mouse)
6. Conjugation Protocol

1. Before adding the antibody to the HRP Mix, add 1 µl of Modifier reagent to each 10 µl of antibody to be labelled. Mix gently.

2. Remove cap from vial of HRP Mix and pipette the antibody sample (with added Modifier) directly onto the lyophilized material. Resuspend gently by withdrawing and re-dispensing the liquid once or twice using a pipette.

3. Replace cap on the vial and leave standing in the dark at room temperature (20-25°C) for 3 hours. Conjugations can also be set up and left overnight; longer incubation times have no negative effect on the conjugate.

4. After incubating for 3 hours (or more), add 1 µl of Quencher reagent for every 10 µl of antibody used and mix gently. The conjugate can be used after 30 minutes. The conjugates do not require purification.

Disclaimer

Labeling of the antibody will not work if the conjugation blocks the active paratope. This situation is rare.
7. Storage of conjugates

Storage at 4°C is recommended for any conjugate. A preservative may be desirable for long-term storage. Other storage conditions may also be satisfactory. The best conditions for any particular conjugate must be determined by experimentation.

Ideally, the antibody to be labeled should be in 10-50mM amine-free buffer pH range 6.5 to 8.5. However, many buffers outside these limits of concentration and pH can be accommodated. Modest concentrations of Tris buffer are also tolerated.

Amine-free buffers, including MES, MOPS, HEPES and phosphate are compatible if they are in the concentration range 10-50mM and have pH values in the range 6.5-8.5, as the addition of Modifier provides the conditions necessary for efficient conjugation. Common non-buffering salts (e.g. sodium chloride), chelating agents (e.g. EDTA), and sugars may be present, as they have no effect on conjugation efficiency. Azide (0.02-0.1%) has little or no effect. If the amine-free buffer is relatively concentrated and outside the pH range 6.6-8.5 you may need to add more modifier for each 10µl of antibody. Excess Modifier is provided so that you can check the pH of the buffer after the addition of the modifier. Ideally the pH should be around 7.3-7.6, though efficient conjugation occurs anywhere between pH 6.8 and 7.8. Avoid buffer components that are nucleophilic, as these may react with the conjugation reaction chemicals. If your buffer contains primary amines
(e.g. amino acids, ethanolamine) and/or thiols (e.g. mercaptoethanol, DTT), you should consider using our Concentration and Purification Kits (ab102778 or ab102784). (Note: Unusually, for an amine, Tris has little effect on conjugation efficiency as long as the concentration is 20mM or less).
8. General FAQs

1. What is the optimal starting concentration for the antibody?

The antibody conjugation kit allows antibody labeling to be performed on a microgram to milligram scale. The amount of antibody should correspond to mass ratios between 1:4 and 1:1 of antibody to conjugate. Based on their molecular weights (e.g. 160kDa for the antibody versus 40kDa for the conjugate), 100-400μg of conjugate can be added to 100μg of antibody. Antibody concentrations of 0.5-5mg/ml give optimal results. We recommend using 10μl, 100μl and 1ml of antibody solution with the 10μg, 100μg and 1mg kit formats, respectively. The antibody concentration for each conjugation kit has been optimised. Please refer to the relevant datasheet or protocol for the recommended antibody concentration.

2. Do I need to purify the antibody before using the conjugation kit?

Yes. The antibody labeling chemistry involves free amine groups. Most proteins/peptides have lysine and/or alpha-amino groups, therefore, any protein/peptide present in the solution will also be labeled. We recommend purifying your antibodies before performing the conjugation. Ascites fluid, serum or hybridoma
culture media should be avoided. View compatible and incompatible buffers in question 5.

3. **Are conjugation kits suitable for proteins and secondary antibodies?**

Yes. The labeling chemistry involves free amines present in lysines and at the N-terminus of a protein. All antibodies have multiple free amine groups and most proteins have lysine and/or alpha-amino groups. As long as lysines are present, secondary antibodies and proteins will be labeled with the conjugation kits. However, they have not yet been specifically tested with secondary antibodies and proteins.

4. **What buffers can be used?**

We recommend using Hepes, MES, MOPS and phosphate-based buffers or any other amine-free buffer. Conjugation reactions can also be prepared in the presence of up to 20mM Tris buffer with almost no reduction in coupling efficiency. Once the reaction is complete, the conjugated antibody can be diluted in any buffer compatible with both label and antibody.

5. **Which buffer additives can be used and what should be avoided?**

Additives such as salts (e.g. NaCl), sugars (e.g. sucrose) and chelators (e.g. EDTA) have no effect on the labeling reaction. We
recommend avoiding nucleophiles such as amino acids (e.g. glycine), blockers (e.g. ethanolamine) and thiols (DTT, mercaptoethanol) that might deactivate the chemical which covalently links the conjugate to the antibody.

**Compatible additives:**

- up to 20mM Tris
- up to 0.5% BSA
- up to 0.1% gelatin
- up to 0.1% sodium azide
- PBS pH7.4
- up to 50% glycerol
- 0.15M sodium chloride
- 50mM HEPES
- 0.02M potassium phosphate
- 0.001% Tween
- Proclin 300
- 5% Trehalose

**Incompatible additives:**

- 60mM citrate + 150mM Tris pH7.8
- Urea
- 50mM Imidazole
- Glycine
- Ethanolamine
- DTT
- Mercaptoethanol
6. **How do I remove additives from the antibody storage buffer?**

   Our Antibody Concentration and Purification kits remove additives with ease and provide a ready-to-use antibody solution compatible with the conjugation kit.

   The Antibody Concentration kit allows an easy concentration and reduction of azide, glycine and Tris.

   The Antibody Purification Kit quickly removes BSA, glycine, Tris, azide etc. and can also be used to purify antibodies from ascites fluid or immune serum.

   For further FAQs visit: www.abcam.com/conjugationFAQS
9. Publications using Conjugation kits

Label: Cy3®  Application: FRET
Effects of pH on molecular mechanisms of chitosane-integrin interactions and resulting tight-junction disruptions

Label: R-PE  Application: Immunocytochemistry and In-vivo imaging*
Intraoperative Imaging of Metastatic Lymph Nodes Using a Fluorophore-conjugated Antibody in a HER2/neu-expressing Orthotopic Breast Cancer Mouse Model

*N.B. No product warranty for this application

Label: APC/Cy7®  Application: Flow cytometry
APR-246/PRIMA-1\textsuperscript{MET} rescues epidermal differentiation in skin keratinocytes derived from EEC syndrome patients with p63 mutations

Label: FITC  Application: Flow cytometry and cellular assay
Constitutive dimerization of glycoprotein VI (GPVI) in resting platelets is essential for binding to collagen and activation in flowing blood.

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